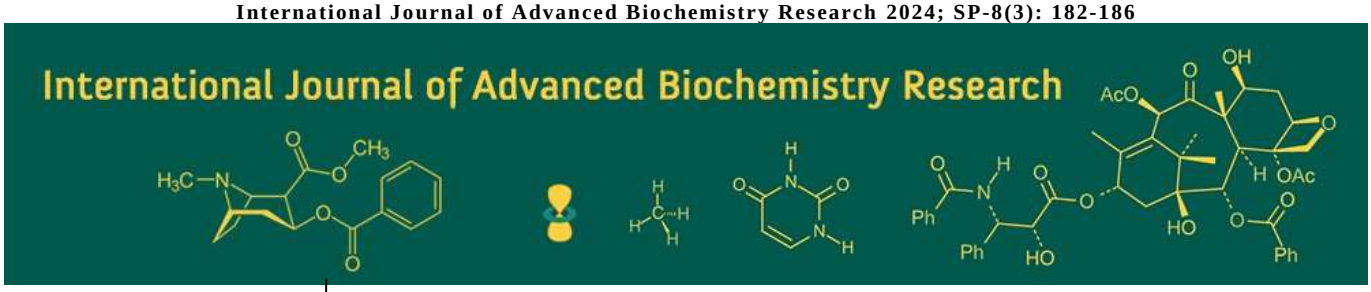

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(3): 182-186
www.biochemjournal.com
Received: 24-12-2023
Accepted: 29-01-2024

Punit Jhandai
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Dinesh Mittal
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Renu Gupta
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Manesh Kumar
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Corresponding Author:
Punit Jhandai
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Phylogrouping of *Escherichia coli* strains from colibacillosis affected chicken

Punit Jhandai, Dinesh Mittal, Renu Gupta and Manesh Kumar

DOI: https://doi.org/10.33545/26174693.2024.v8.i3Sc.718

Abstract
Avian colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), contributes significantly to economic losses within the poultry sector, including mortality, morbidity, and carcass condemnation. Various strains of *E. coli*, categorized into different phylogroups, possess unique genetic and phenotypic characteristics that influence their ability to cause disease. Present study was undertaken to determine the phylogroups of *Escherichia coli* isolates isolated from colibacillosis affecting poultry. Tissue samples were collected from poultry flocks exhibiting typical colibacillosis lesions like pericarditis, perihepatitis, air sacculitis etc. In this study, 50 *E. coli* isolates, consisting of 45 APEC and 5 non-APEC isolates, were categorized into phylogroups. The primer pairs *yjaA, chuA, arpA, TspE4.C2* and *trpA* were utilized for PCR to amplify specific fragments, enabling phylogenetic typing. The results classified *E. coli* isolates (n=50) into B1 (31%), A (28%), B2 (10%), F (6%) and D (4%). Four *E. coli* isolates (8%) could not be classified or were of unknown type, and none of the *E. coli* isolates fell into phylogroup C. Approximately 6% of the *E. coli* isolates were assigned to either Clade I or Clade II. The research showed that phylogroup B1 is prevalent in *E. coli* found in colibacillosis-infected poultry in Haryana. The presence of unidentified phylogroups emphasizes further investigation in the protocols and techniques used for phylogrouping. This might suggest possibility for discovering new phylogroups.

Keywords: Phylogrouping, *Escherichia coli* strains, colibacillosis affected chicken

Introduction
Poultry, particularly chicken, is a widely consumed food globally. The poultry sector in India has experienced swift growth since the late 1970s (Muduli *et al.*, 2019) [18]. However, this sector faces ongoing risks from a variety of infectious illnesses instigated by bacteria, fungi, viruses, and parasites such as colibacillosis, salmonellosis, campylobacteriosis, avian influenza, coccidiosis, and more. (Cutler, 2002) [5]. Of these infections, colibacillosis stands out as a primary cause of fatalities, resulting in notable economic losses and the rejection of carcasses (Huff *et al.*, 2002) [10]. It includes any localized or widespread infection initiated by avian pathogenic *Escherichia coli* (APEC), includes conditions like colisepticemia, chronic respiratory disease, coliform cellulitis, venereal colibacillosis, and peritonitis. The bacterium APEC contributes significantly to economic losses in the poultry industry due to mortality, morbidity, carcass condemnation, and expenses associated with treatment and disinfection (Kathayat *et al.*, 2021) [13]. Furthermore, a considerable amount of *Escherichia coli* strains that cause intestinal infections have the potential to transmit to other animals or humans, whether through direct or indirect means, thereby posing a notable risk to both animal and human well-being (Doyle and Erickson, 2006) [7]. Avian pathogenic *E. coli* can be categorized according to its virulence factors, serotype, phylogenetic group, and resistance to drugs. The genetic makeup of *E. coli* strains often experiences changes like additions, deletions, and recombinations in response to natural selection, resulting in divergence (Cordoni *et al.*, 2016, O'Boyle *et al.*, 2020) [4, 20]. As a result, *E. coli* displays a wide array of genetic substructures, with at least eight different phylogenetic groups divided into 3 clusters: clusters containing phylogroups B2, F, and G, another grouping phylogroups A, C, B1, and E, and finally phylogroup D in a separate cluster (Clermont *et al.*, 2013, Clermont *et al.*, 2019, Gonzalez-Alba *et al.*, 2019, Denamur *et al.*, 2021) [2, 3, 9, 6]. The revised Clermont classification expands on the prior categorization of four phylogroups (A, B2, B1, and D).

It employs a set of phylogenetic markers *viz*. *yjaA*, *chuA*, DNA fragment *arpA*, *TspE4.C2*, *trpA*, and *ArpAgpE* to amplify particular fragments via PCR for target specificity (Clermont *et al.*, 2013) [2].

Materials and Methods

Samples collection and processing

The tissue samples were collected from chicken affected from colibacillosis from in and around Hisar district. Isolation was conducted utilizing conventional techniques involving MacConkey agar, brain heart infusion broth, EMB agar, Gram's staining, and subsequent confirmation through PCR amplification of the *PhoA* gene (Jhandai *et al.*, 2019) [11]. In the study, a combined total of 50 *E. coli* isolates, classified as either APEC (n=45) or non-APEC (n=5), were employed for phylogroup examination.

DNA extraction

The genomic DNA isolation procedure was conducted utilizing the boiling/snap chill method (Englen and Kelley, 2000) [8] with slight modification. In summary, a loopful of colonies was suspended in 100µl of TE buffer (pH-8.3) and centrifuged at 1000 rpm for 3 minutes. Subsequently, the suspensions were boiled at 100 °C for 10-12 minutes, promptly cooled on ice, and centrifuged at 10000 rpm for 10-12 minutes. The resulting supernatant was then transferred to clean nuclease-free tubes and stored at -20 °C until further utilization.

Phylogenetic Typing of *E. coli* Isolates using PCR

All *E. coli* isolates underwent phylogenetic typing following the procedures outlined in the methods described by Clermont *et al.* (2013) [2]. The primer pairs used for phylogenetic typing were *chuA*, *yjaA*, *TspE4.C2*, *arpA*, *ArpAgpE* and *trpA* (Table 3.1). The PCR procedure was conducted in a reaction volume of 25 µl, under the following PCR conditions: initial denaturation for 4 min at 94 ℃ followed by 30 cycles of denaturation for 5 s at 94 ℃; annealing for 30 s each at 52 ℃, 60 ℃ and 62 ℃ for group E, quadruplex and group C, respectively; and extension for 30 s at 72 ℃ with a final extension at 72 ℃ for 5 min. The PCR products were observed through horizontal gel electrophoresis utilizing a 1.5% agarose gel. A 100 bp molecular weight marker served as the reference standard for size. Following electrophoresis, the gels were stained with ethidium bromide, and the bands representing each gene were captured digitally using a gel documentation system.

Table 1: Primers used in the phylogenetic typing PCR assays

Gene	Primers used	Primer's sequence	Amplicons size (base pairs)	Reference
Quadruplex	*chuA*	F-5′-ATGGTACCGGACGAACCAAC-3′	288	Clermont *et al.*, 2013 [2]
		R-5′-TGCCGCCAGTACCAAAGACA-3′		
	yjaA	F-5′-CAAACGTGAAGTGTCAGGAG-3′	211	
		R-5′-AATGCGTTCCTCAACCTGTG-3′		
	TspE4.C2	F-5′-CACTATTCGTAAGGTCATCC-3′	152	
		R-5′-AGTTTATCGCTGCGGGTCGC-3′		
	arpA	F-5′-AACGCTATTCGCCAGCTTGC-3′	400	
		R-5′-TCTCCCCATACCGTACGCTA-3′		
Group E	*ArpAgpE*	F-5′-GATTCCATCTTGTCAAAATATGCC-3′	301	Lescat *et al.*, 2013
		R-5′-GAAAAGAAAAAGAATTCCCAAGAG-3′		
Group C	*trpA*	F-5′-AGTTTTATGCCCAGTGCGAG-3′	219	
		R-5′-TCTGCGCCGGTCACGCCC-3′		

Results and Discussion

Write conclusion in 100-120 following

The classification of all 50 *E. coli* isolates into phylogenetic groups using *chuA* (288bp), *yjaA* (211bp), *TspE4.C2* (152bp), *arpA* (400bp), *ArpAgpE* and *trpA* genes primers indicated that phylogroup B1 (38%) was dominant group followed by A (28%), B2 (10%), unknown/untypable group (8%), F, Clade I/II (6% each) and D (4%) (Fig. 4.1). Similar results were also obtained during quadreplex PCR (Fig. 4.2). In this study, none of the *E. coli* isolates were categorized under phylogroup C. The allocation and prevalence of various phylogroups among both APEC and non-APEC isolates are outlined in Table 4.1. Notably, significant variations in the distribution of virulence traits were observed among phylogenetic groups, despite the isolates being recovered from similar infection types. Comparable findings have been documented by Rodriguez-Siek *et al.* (2005) [21] and Mittal *et al.* (2022) [17], wherein phylogroup A is dominating phylogroup among APEC isolates. Phylogroup B2 was significantly associated with non-APEC isolates in the present study. Out of the 50 *E. coli* isolates obtained from poultry lesions afflicted with colibacillosis, phylogroup B1 was the most common (19 isolates), followed by A (14), B2 (5), F (3), F (3) and D (2). In the current investigation, none of the isolates fall within phylogroups C and E. Most APEC isolates are categorized under phylogenetic types A, B1, and D, whereas the majority of human ExPEC isolates primarily belong to phylogenetic types B2, and to a lesser degree, D (Rodriguez-Siek *et al.*, 2005) [21]. Further, the pathogenicity is caused by plasmid-mediated pathogenicity-associated islands (PAIs) and other mobile and extrachromosomal elements. It cannot be accounted for phylogenetic categorization, which depends on recognizing specific chromosomal markers. The presence of extra-chromosomally positioned PAIs is a characteristic trait of the APEC pathotype and appears to play a crucial role in APEC virulence (Tivendale *et al.*, 2004; Johnson *et al.*, 2008; Skyberg *et al.*, 2008) [23, 12, 22]. However, these isolates were obtained from chickens afflicted with perihepatitis and other colibacillosis syndromes (Logue *et al.*, 2017) [16]. Phylogroups A and B1 have been recognized as closely related (Lecointre *et al.*, 1998) [14]. Murase and Ozaki (2022) [19] proposed that *E. coli* isolates classified under phylogroups A and B1, derived from colibacillosis lesions, exhibit pathogenic potential as indicated by virulence genotyping. The findings of this study are different from the previous findings by Murase and Ozaki, 2022 [19] who found

37 isolates belonged to phylogroup F recovered from 56 colibacillosis-infected broiler birds. It may be attributed to geographical differences and/or any other selection pressure due to climatic conditions.

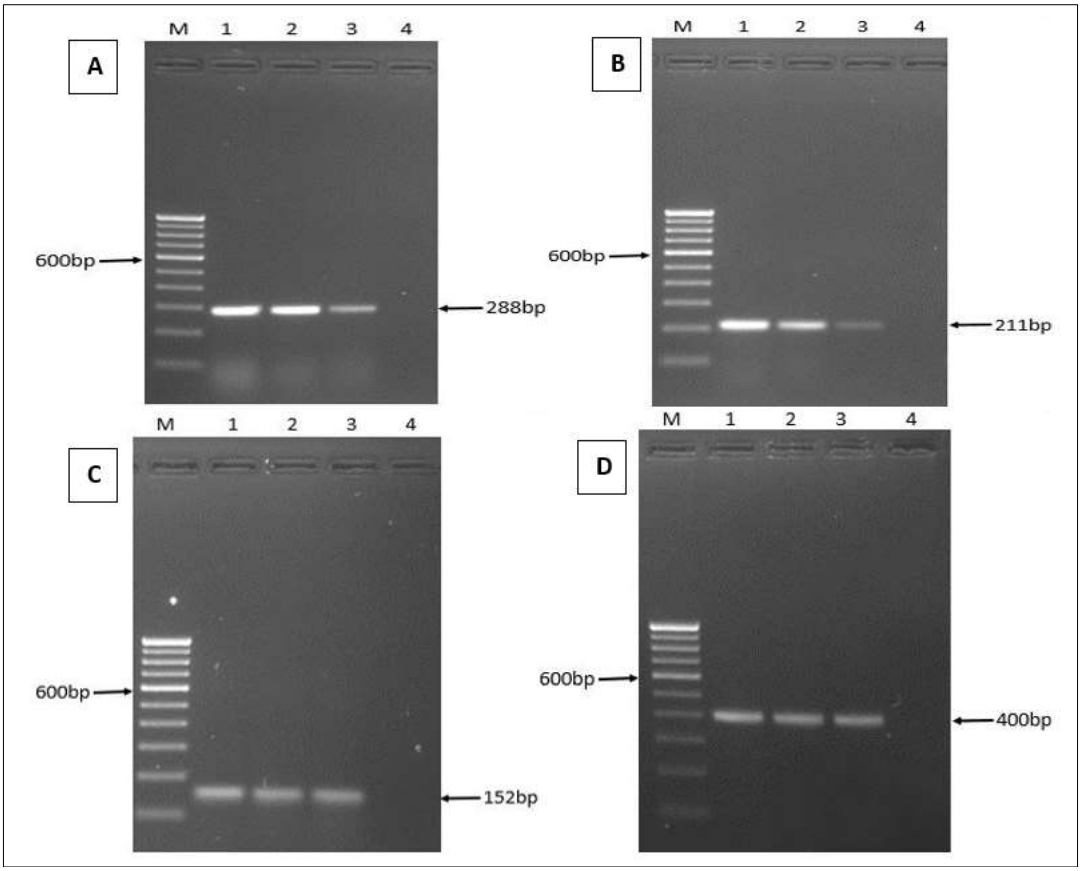

Fig 1: Phylogenetic typing of *E. coli* isolates using A) *chuA* (288bp); B) *yjA* (211bp); C) *TspE4.C2* (152bp); D) *arpA* (400bp) genes by PCR Lane M– 100bp DNA ladder; Lanes 1-2– *E. coli* isolates; Lanes 3– Positive control; Lane 4– Negative control

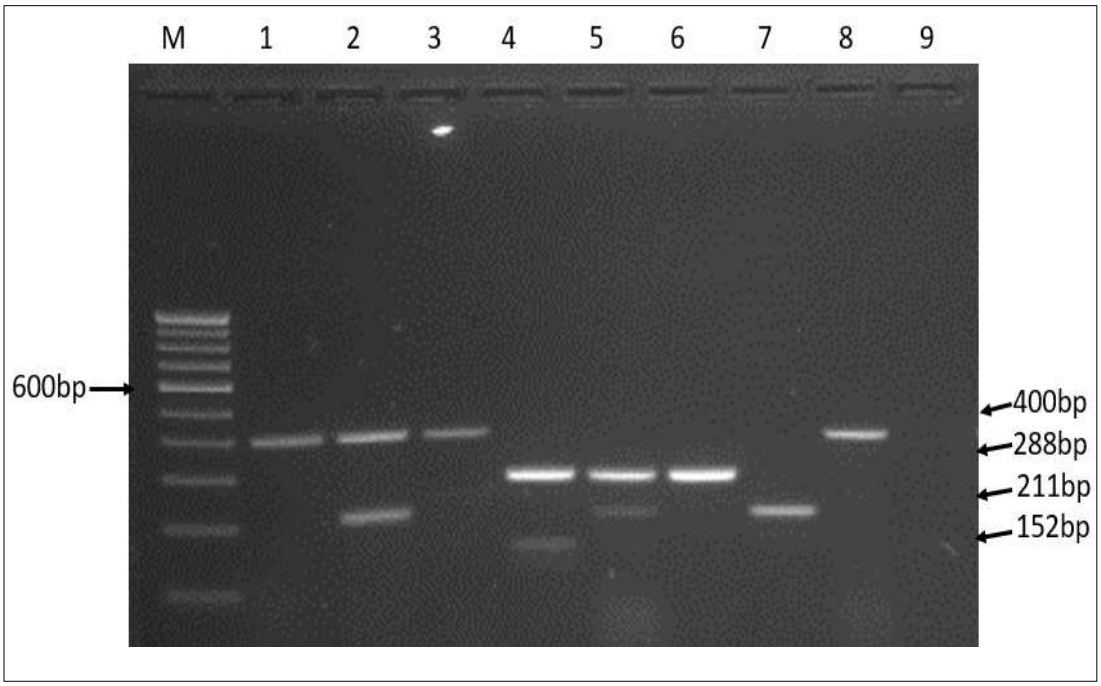

Fig 2: Phylogenetic typing of *E. coli* isolates using quadruplex phylogroup genes by PCR Lane M– 100bp DNA ladder; Lanes 1-8– *E. coli* isolates with different gene combination; Lane 9– Negative control

Table 2: Association of genes (*arpA, chuA, yjA, TspE4.C2*) and phylogroups

Phylogroup/Category/Clade	Genotype combination	Number of isolates
A (14)	*arpA* + *chuA* - *yjA* + *TspE4.C2* -	9
	arpA + *chuA* - *yjA* - *TspE4.C2* -	5
B1 (19)	*arpA* + *chuA* - *yjA* - *TspE4.C2* +	19
B2 (5)	*arpA* - *chuA* + *yjA* + *TspE4.C2* +	5
	arpA - *chuA* + *yjA* - *TspE4.C2* +	0
Clade I/II (3)	*arpA* - *chuA* - *yjA* + *TspE4.C2* -	3
D (2)	*arpA* + *chuA* + *yjA* - *TspE4.C2* +	2
F (3)	*arpA* - *chuA* + *yjA* - *TspE4.C2* -	3
Unknown (4)	*arpA* + *chuA* - *yjA* + *TspE4.C2* +	4

Conclusion

In conclusion, the phylogenetic analysis of 50 E. coli isolates using multiple gene markers revealed a diverse distribution among phylogroups, with B1 being the most prevalent followed by A, B2, and other minor groups. Interestingly, no isolates were categorized under phylogroup C. These findings align with previous studies, highlighting the dominance of phylogroup A among APEC isolates. Conversely, phylogroup B2 was notably associated with non-APEC strains. Moreover, the distribution of virulence traits varied significantly among phylogenetic groups, underscoring the complexity of *E. coli* pathogenicity. Despite the absence of phylogroups C and E in this investigation, the presence of pathogenicity-associated islands (PAIs) suggests a crucial role in APEC virulence. Geographical variations and selection pressures may account for discrepancies in phylogenetic composition observed across studies. Overall, this study enhances our understanding of *E. coli* pathogenesis and underscores the importance of molecular typing in epidemiological investigations.

Acknowledgements

The authors express their gratitude to the Head of the Division of Veterinary Public Health and Epidemiology at Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS) for providing the essential facilities to conduct this study.

References

1. Beghain J, Bridier-Nahmias A, Le Nagard H, Denamur E, Clermont O. Clermont Typing: an easy-to-use and accurate in silico method for *Escherichia* genus strain phylotyping. Microb Genom; c2018, 4(7).
2. Clermont O, Christenson JK, Denamur E, Gordon DM. The Clermont *Escherichia coli* phylotyping method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep. 2013;5(1):58-65.
3. Clermont O, Dixit OV, Vangchhia B, Condamine B, Dion S, Bridier-Nahmias A, *et al*. Characterization and rapid identification of phylogroup G in *Escherichia coli*, a lineage with high virulence and antibiotic resistance potential. Environ Microbiol. 2019;21(8):3107-17.
4. Cordoni G, Woodward MJ, Wu H, Alanazi M, Wallis T, La Ragione RM. Comparative genomics of European avian pathogenic E. coli (APEC). BMC Genomics. 2016;17(1):1-21.
5. Cutler GJ. Diseases of the chicken. In: Commercial Chicken Meat and Egg Production. Springer, Boston, MA; c2002. p. 473-542.
6. Denamur E, Clermont O, Bonacorsi S, Gordon D. The population genetics of pathogenic *Escherichia coli*. Nat Rev Microbiol. 2021;19(1):37-54.
7. Doyle MP, Erickson MC. Reducing the carriage of foodborne pathogens in livestock and poultry. Poult Sci. 2006;85:960-973.
8. Englen MD, Kelley LC. A rapid DNA isolation procedure for the identification of *Campylobacter jejuni* by the polymerase chain reaction. Lett Appl Microbiol. 2000;31:421-426.
9. Gonzalez-Alba JM, Baquero F, Canton R, Galan JC. Stratified reconstruction of ancestral *Escherichia coli* diversification. BMC Genomics. 2019;20(1):1-5.
10. Huff WE, Huff GR, Rath NC, Balog JM, Xie H, Moore Jr PA, *et al*. Prevention of *Escherichia coli* respiratory infection in broiler chickens with bacteriophage (SPR02). Poult Sci. 2002;81(4):437-441.
11. Jhandai P, Kumar A, Vaishali, Gupta R. Detection of *Escherichia coli* from food of animal origin in Hisar district of Haryana. Har Vet. 2019;58(2):245-247.
12. Johnson TJ, Wannemuehler Y, Doetkott C, Johnson SJ, Rosenberger SC, Nolan LK. Identification of minimal predictors of avian pathogenic *Escherichia coli* virulence for use as a rapid diagnostic tool. J Clin Microbiol. 2008;46(12):3987-3996.
13. Kathayat D, Lokesh D, Ranjit S, Rajashekara G. Avian Pathogenic *Escherichia coli* (APEC): An overview of virulence and pathogenesis factors, zoonotic potential, and control strategies. Pathogens. 2021;10(4):467. DOI: 10.3390/pathogens10040467.
14. Lecointre G, Rachdi L, Darlu P, Denamur E. *Escherichia coli* molecular phylogeny using the incongruence length difference test. Mol Biol Evol. 1998;15(12):1685-1695.
15. Lescat M, Clermont O, Woerther PL, Glodt J, Dion S, Skurnik D, *et al*. Commensal *Escherichia coli* strains in Guiana reveal a high genetic diversity with host-dependant population structure. Environ Microbiol Rep. 2013;5(1):49-57.
16. Logue CM, Wannemuehler Y, Nicholson BA, Doetkott C, Barbieri NL, Nolan LK. Comparative analysis of phylogenetic assignment of human and avian expec and fecal commensal *Escherichia coli* using the (previous and revised) clermont phylogenetic typing methods and its impact on avian pathogenic *Escherichia coli* (APEC) classification. Front Microbiol. 2017;8:283. DOI: 10.3389/fmicb.2017.00283.
17. Mittal D, Grakh K, Kumar M, Prakash A, Kumar R, Kumar P. Extended phylogrouping of pathogenic and non- pathogenic *Escherichia coli* isolates of avian origin. J Pharm Innov. 2022;11:265-269.

18. Muduli S, Champati A, Popalghat HK, Patel P, Sneha KR. Poultry waste management: An approach for sustainable development. Int J Adv Sci Res. 2019;4:08-14.
19. Murase T, Ozaki H. Relationship between phylogenetic groups of *Escherichia coli* and pathogenicity among isolates from chickens with colibacillosis and healthy chickens. Poult Sci. 2022;101(9):102007. DOI: 10.1016/j.psj.2022.102007.
20. O'Boyle N, Connolly JP, Tucker NP, Roe AJ. Genomic plasticity of pathogenic *Escherichia coli* mediates D serine tolerance via multiple adaptive mechanisms. Proc Natl Acad Sci. 2020;117(36):22484-93.
21. Rodriguez-Siek KE, Giddings CW, Doetkott C, Johnson TJ, Fakhr MK, Nolan LK. Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. Microbiology. 2005;151(6):2097-2110.
22. Skyberg JA, Johnson TJ, Nolan LK. Mutational and transcriptional analyses of an avian pathogenic *Escherichia coli* CoIV plasmid. Microbiol. 2008;8:10-18.
23. Tivendale KA, Allen JL, Ginns CA, Crabb BS, Browning GF. Association of iss and iucA, but not tsh, with plasmid-mediated virulence of avian pathogenic *Escherichia coli*. Infect Immun. 2004;72(11):6554-6560.